

TOYOPEARL® ORIENTATION SHEET

HIC

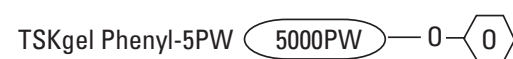
RESINS	Pore Size
Ether-650 (S, M)	1,000 Å
PPG-600M	750 Å
Phenyl-650 (S, M, C)	1,000 Å
Phenyl-600M	750 Å
Butyl-650 (S, M, C)	1,000 Å
Butyl-600M	750 Å
SuperButyl-550C	500 Å
Hexyl-650C	1,000 Å

S = 35 µm, M = 65 µm, C = 100 µm

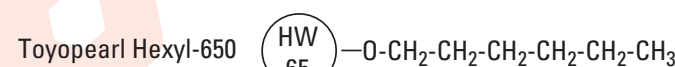
Toyopearl Hydrophobic Interaction Resins provide exceptional selectivities of proteins by recognition of their surface hydrophobicities. The gentle, non-denaturing characteristics of HIC retains high levels of protein activity. Five different ligands provide a complete range of selectivities for optimization of retention, resolution, and recovery. Various pore sizes allow for optimization of dynamic capacity for individual target proteins.

Suggested Use: The protein with the lowest hydrophobicity should be bound to the most hydrophobic media and vice versa (see retention bar chart). Butyl-600, PPG-600 and Phenyl-600 are optimized for mAb purification by combining very high DBC with high recovery.

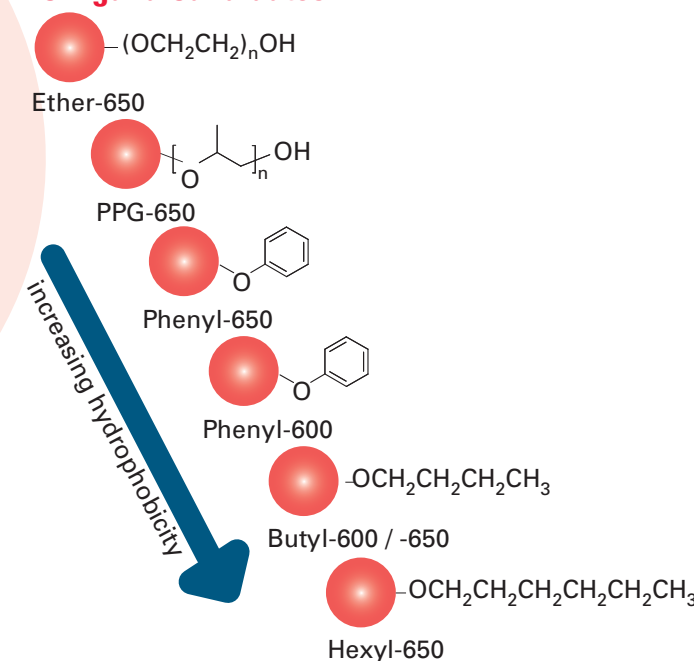
Structure of TSK-GEL HIC Resins



Structure of Toyopearl HIC Resins



HIC Ligand Candidates



Adsorption Capacity

Resins	Lysozyme (mg/ml resin)	Other Proteins (mg/ml)
Ether-650 (S, M)	10-30 ¹⁾	-
PPG-600M	45-55 ¹⁾	mAb (Anti-LH): > 30 ²⁾
Phenyl-650 (S, M, C)	30-50 ¹⁾	mAb (Anti-LH): > 30 ²⁾
Butyl-600M	-	mAb (Anti-LH): > 50 ²⁾
Butyl-650 (S, M, C)	40 ¹⁾ , 40 ²⁾	h-mAb: ≈ 40
SuperButyl-550C	30 - 50 ¹⁾ , 61 ²⁾	-
Hexyl-650C	30 - 50 ¹⁾	-
Phenyl-600M	60 ¹⁾	h-mAb: > 50 ¹⁾

¹⁾Static ²⁾Dynamic at 10 % Breakthrough

IEC

ANION EXCHANGERS	Pore Size
GigaCap Q-650M	400 Å
SuperQ-650 (S, M, C)	750 Å
Q-600C AR	500 Å
QAE-550C	1,000 Å
DEAE-650 (S, M, C)	1,000 Å

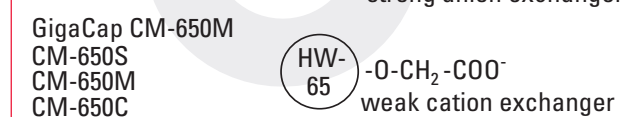
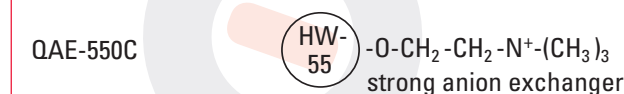
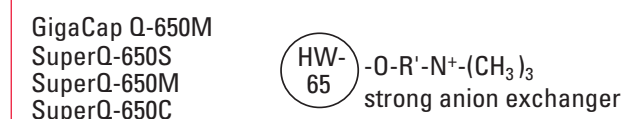
S = 35 µm, M = 65 µm, C = 100 µm

CATION EXCHANGERS

ANION EXCHANGERS	Pore Size
GigaCap S-650M	1,000 Å
GigaCap CM-650M	1,000 Å
CM-650 (S, M, C)	1,000 Å
SP-650 (S, M, C)	500 Å
SP-550C	500 Å
MegaCap II SP-550EC	500 Å

S = 35 µm, M = 65 µm (GigaCap M = 75 µm), C = 100 µm, EC = 200 µm

Structure of Toyopearl IEC Resins



Adsorption Capacity

	GigaCap Q-650	Super Q-650	DEAE-650	QAE-550	Q-600C AR	GigaCap S-650	MegaCap II SP-550	SP-550	SP-650	GigaCap CM-650	CM-650
BSA	>160 ¹⁾ ; >150 ²⁾	≈ 145 ²⁾	≈ 25 ²⁾	70 ¹⁾ ; 30 ²⁾	>120 ¹⁾ ; ≈ 100 ²⁾	-	-	-	-	-	-
Lysozyme	-	-	-	-	-	>200 ¹⁾	≈ 70 ²⁾	110 ¹⁾ ; 70 ²⁾	50 ¹⁾ ; 35 ²⁾	38 ¹⁾ ; 35 ²⁾	35 ²⁾
mAb	108 ²⁾	13 ²⁾	≈ 31 ²⁾	32 ²⁾	90 ²⁾	150 ¹⁾ ; 145 ²⁾	-	14 ²⁾	12 ²⁾	>110 ¹⁾ ; ≈ 100 ²⁾	-
Insulin	-	-	-	-	-	≈ 140 ²⁾	>100 ¹⁾ ; ≈ 80 ²⁾	≈ 80 ²⁾	≈ 50 ²⁾	-	-

¹⁾Static ²⁾Dynamic at 10 % Breakthrough

Toyopearl Ion Exchange Resins. IEC is the most common liquid chromatographic method used in manufacturing of biopharmaceuticals. Toyopearl IEC resins have the advantages of the HW-65 or HW-55 base matrices. For example, high permeability enables rapid pH or ionic strength equilibration, often within only three column volumes.

Suggested Use: All functionalities for recombinant and membrane proteins. SP-650, CM-650 for mAbs, DEAE-650 for blood products and nucleic acids, SuperQ optimized for proteins <50 kDa. MegaCap II, SP-550, Q-600C AR and especially GigaCap S, Q and CM have very high dynamic capacities and enable high throughput for concentrating dilute process streams.

SEC

RESINS	Pore Size
HW-40 (S, F, C)	50 Å
HW-50 (S, F)	125 Å
HW-55 (S, F)	500 Å
HW-65 (S, F, C)	1,000 Å
HW-75 (F)	> 1,000 Å

S = 30 µm, F = 45 µm, C = 75 µm

Toyopearl Size Exclusion Resins separate molecules according to their physical size. They are ideal for fractionation of complex samples, buffer change or the final polishing step of a purification protocol.

Suggested Use: HW-40: fraction of low MW compounds or buffer exchange; HW-50: for peptide fragments; HW-55, HW-65: for proteins; HW-75: for large nucleic acids or plasmid DNA.

HW resins are excellently suited for the separation of oligosaccharide or glycosylated proteins.

Molecular Weight operating Ranges of SEC Media

Resin Type	Polyethyleneglycols	Dextrans	Globular Proteins
HW-40	100 - 3,000	100 - 7,000	100 - 10,000
HW-50	100 - 18,000	500 - 20,000	500 - 80,000
HW-55	100 - 150,000	1,000 - 200,000	1,000 - 700,000
HW-65	500 - 1,000,000	10,000 - 1,000,000	40,000 - 5,000,000
HW-75	4,000 - 5,000,000	100,000 - 10,000,000	500,000 - 50,000,000

Toyopearl resins are hydrophilic, macroporous, bulk bioprocessing media, made exclusively for large-scale chromatographic applications. Because of their polymeric backbone structure, the rigid Toyopearl packings assure excellent pressure/flow characteristics (1000 cm/h, 5 bar). The media are stable over the pH 2.0 - 13.0 range for normal operating conditions and pH 2.0 - 14.0 range for cleaning conditions.

The particle sizes are 20 - 50 µm superfine grade for the highest performance, 40 - 90 µm medium grade for

AFC

REACTIVE RESINS	Pore Size
AF-Amino-650M	1,000 Å
AF-Carboxy-650M	1,000 Å
AF-Formyl-650M	1,000 Å

ACTIVATED RESINS

ACTIVATED RESINS	Pore Size
AF-Epoxy-650M	1,000 Å
AF-Tresyl-650M	1,000 Å

READY TO USE RESINS

READY TO USE RESINS	Pore Size
AF-BlueHC-650M	1,000 Å
AF-Chelate-650M	1,000 Å
AF-HeparinHC-650M	1,000 Å
AF-Red-650ML	1,000 Å

M = 65 µm ML = 65 µm

Toyopearl Affinity Resins are mechanical stable and provide good pH stability and high ligand capacities. Activated resins are supplied as freeze-dried powders (1g/4ml gel), while reactive and ready to use resins are supplied as preswollen gels.

Suggested Use: Epoxy-650 readily forms a stable ether linkage to small ligands, Tresyl-650 is recommended for direct coupling of proteins with mild conditions. The reactive Amino, Carboxy, or Formyl-650 immobilize either proteins or small ligands by the carbodiimide or reductive alkylation coupling methods. BlueHC-650 is mainly used for purification of recombinant HSA and EPO, whereas Red-650 is useful in the purification of nucleotide-dependent enzymes. Chelate-650 will be used in the IMAC-mode for the purification of HIS-tagged proteins. HeparinHC-650 interacts with a wide range of plasma components, e.g. DNA polymerase, Factor VIII and IX.

Resin	Target Ligand	Coupling Cond.	Coupling Agent	Human IgG*	Lysozyme*
Epoxy	R-NH ₂	pH: 9.0-11.0, 40 °C	none		
	R-SH	pH: 7.0-8.0, 25 °C	none		
	R-OH	0.1N NaOH, 40 °C	none		
Tresyl	R-NH ₂ , R-SH	pH: 7.5-8.0, 25 °C	none	10.0	60.0
Amino	R-COOH, R-CHO	pH: 4.0-6.0/7.0, 25 °C	EDC**/NaCNBH ₃	6.7	5.8
Carboxy	R-NH ₂	pH: 4.0-6.0, 25 °C	EDC**	11.7	17.5
Formyl	R-NH ₂	pH: 7.0, 25 °C	NaCNBH ₃	15.0	20.0

* Coupling Densities (mg/ml) ** N'-[3-Dimethylaminopropyl]-N-ethylcarbodiimide

economical purification, and 50 - 150 µm coarse grade for capture chromatography. The large pore size insures high capacity for high molecular weight molecules, and faster separation and recycling times. Toyopearl media are available for Size Exclusion, Ion Exchange, Hydrophobic Interaction, and Affinity Chromatography in large-scale processes. For most resins small pre-packed columns, the ToyoScreen® columns are offered for fast and easy resin screening.